Nutritional and Phytochemical Content of High-Protein Crops

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ABSTRACT

Sustainable sources of high-protein plants could help meet future protein requirements. Buckwheat, green pea, fava bean, hemp and lupin were analyzed by proximate analysis and ICP-MS to determine the macro- and micronutrient content and LC-MS to elucidate the phytochemical profiles. The protein content ranged from 20-43 % (w/w) and all were found to be rich in insoluble fibre; 9-25 % (w/w). The selected crops had a favourable micronutrient profile with phosphorous levels ranging from 2.22 ± 0.05 g kg^{-1} to 9.72 ± 0.41 g kg^{-1}, while iron levels ranged from 20.23 ± 0.86 mg kg^{-1} to 69.57 ± 7.43 mg kg^{-1}. The crops contained substantial amounts of phytophenolic compounds. In particular, buckwheat was a rich source of pelargonidin (748.17 ± 75.55 mg kg^{-1}), epicatechin (184.1 ± 33.2 mg kg^{-1}), quercetin (35.66 ± 2.22 mg kg^{-1}), caffeic acid (41.74 ± 22.54 mg kg^{-1}) and 3-hydroxyphenylacetic acid (63.64 ± 36.16 mg kg^{-1}), hemp contained p-coumaric acid (84.02 ± 8.10 mg kg^{-1}), cyanidin (58.43 ± 21.01 mg kg^{-1}), protocatechualdehyde (34.77 ± 5.15 mg kg^{-1}) and gentisic acid (31.20±1.67 mg kg^{-1}) and fava bean was the richest source of ferulic acid (229.51 ± 36.58 mg kg^{-1}) and its 5-5´ (39.99 ± 1.10 mg kg^{-1}) and 8-5 dimers (58.17 ± 6.68 mg kg^{-1}). Demonstrating that these crops are rich sources of protein, fibre and phytochemicals could

encourage higher consumption and utilisation as healthy and sustainable ingredients by the food and drink industry.

KEYWORDS: *sustainable and healthy food ingredients; future protein supply; high-protein plants; legumes; food security*

INTRODUCTION

Sourcing sufficient amounts of protein to meet future dietary requirements is a critical issue, which scientists and policy makers are currently addressing worldwide. Indeed a recent report by the European Innovation Partnership 'Agricultural Productivity and Sustainability'[1] identified the need for greater plant based protein production, both for animal feed and increasingly for direct human consumption. There is an accruing body of evidence identifying the urgent need to shift towards a more plant based diet for both environmental and physiological reasons.[2,3] At the moment, the global population is rapidly growing and with it the request of dietary protein, mainly from animal origin, which is projected to increase by more than 50% by 2030 compared to that of 2000.[4] The traditional Western dietary pattern focuses predominantly on animal-based products to satisfy protein requirements, and is environmentally detrimental as it relies on intensive livestock farming which deeply deteriorates the natural resources.[5] It has been estimated that in European Union 27 the total greenhouse gas emissions attributable to the livestock sector are between 12 and 61% of the total anthropogenic emissions.[6] Also, from the physiological standpoint, the high intake of meat, especially red and processed, is associated with higher incidence of coronary heart diseases, diabetes mellitus, and several forms of cancer.[7-9] Greater consumption of dietary protein from sustainable plant sources appears an immediate and effective way to both mitigate the environmental impact of our diet, and to reach and maintain a healthier diet.[10]

The production and consumption of local food, including high protein crops, may contribute towards achieving a sustainable diet, although in high latitude countries such as Scotland, the shift in the agricultural system towards novel protein crop production, first needs an assessment of the feasibility and ability of the agri-environment to deliver appropriate crops of suitable quality and yield. To this end, five high protein crops have been identified that can

53 be or have potential to be sustainably grown in Scotland. These are lupin (*Lupinus albus*),
54 hemp (*Cannabis sativa*), buckwheat (*Fagopyrum esculentum*), green pea (*Pisum sativum*),
55 fava bean (*Vica faba*). These crops offer a valid alternative to importing protein rich crops
56 such as soybean, and contribute to enhance the diversity, and hence the economic stability of
57 local agricultural production. They represent a rich source of energy, provide complex
58 carbohydrates and high quality protein,[11] and are considered important sources of bioactive
59 non-nutrient plant compounds, generally known as phytochemicals.[12,13]

60 Among the large group of phytochemicals, phenolic compounds have been widely
61 investigated due to their ubiquitous presence in plants, and their beneficial biological effects
62 in humans.[14] Phenolic compounds comprise a varied family of molecules derived from the
63 phenylpropanoid pathway, with phenolic acids and flavonoids being the main classes of
64 dietary phenolics in the European population.[15] Phenolic compounds can occur in the free
65 form or bound to other cell wall components.[16] This aspect significantly impacts upon their
66 availability in the body and associated benefits. Flavonoids are a large polyphenolic
67 subgroup, which are normally conjugated to sugar molecules and can be further classified
68 into anthocyanins, flavones, isoflavones, flavanones, flavonols and flavan-3-ols; they are all
69 common dietary components.[17] There are many studies demonstrating the positive effects of
70 a regular consumption of food rich in phenolic compounds.[18,19] These include the effects of
71 polyphenols such as proanthocyanidins and quercetin glycosides from azuki bean seed coats
72 which attenuated blood pressure elevation and ameliorated diabetic nephropathy.[20,21]
73 Nevertheless, proanthocyanidins from fava bean and bird's-foot trefoil have shown to
74 precipitate protein and minerals in the gastrointestinal tract reducing their bioavailability.[22,23]
75 The aim of the present work was to determine the macro- micro- and non- nutrient
76 (predominantly phenolic) profiles of commercially available food grade flours from lupin,
77 hemp, buckwheat, green pea, fava bean and compare them to wheat flour. Furthermore, the

potential dietary role of these crops, which can be considered sources of both sustainable plant protein and bioactive phytochemicals, and the health benefits of plant protein rich diets are discussed.

MATERIALS AND METHODS

Standards, reagents and plant material. Standards and general laboratory reagents were purchased from Sigma-Aldrich (Gillingham, England) and Fisher Scientific UK Ltd. (Loughborough, England). Chemicals used for Inductively-Coupled Mass Spectrometry (ICP-MS) analysis were nitric acid of TraceSelect Ultra grade from Fluka (Gillingham, England), hydrochloric acid (30%) of Ultrapur grade from Merck (Darmstadt, Germany), and deionized water from Millipore (Bedford, USA). Single elements standards were purchased from all Inorganic Ventures (Christiansburg, USA). Commercially available flours were obtained as follows: strong white flour was purchased from Tesco Stores Ltd. (Cheshunt, UK); buckwheat flour was purchased from Arrowhead Millls, Inc. (Melville, USA); hemp flour was purchased from Yorkshire Hemp Ltd. (Driffield, UK); fava bean flour was purchased from The Barry Farm (Wapakoneta, USA); green pea flour was purchased from Bob's Red Mill Natural Foods (Milkwaukie, USA); lupin flour was purchased from Terrena Lup' Ingredients (Martigne Ferchaud, France). All the products were purchased dried and milled. Flours were stored at room temperature apart from green pea flour which has been kept at 4 °C as specified by the manufacturer.

Macronutrient Analysis. Routine proximate analytical procedures were employed to determine the macronutrient composition of the flours. Protein was measured as total nitrogen by the Dumas combustion method using a Vario Max CN analyser and the nitrogen content multiplied by 6.25 to generate the protein concentration[24]. Resistant starch and

nonstarch polysaccharide (NSP) were measured according to the methods described by Englyst et al.[25,26] Total fat was determined by the Soxtec method (Soxtec™ 2050 Auto Fat Extraction System).[27]

Micronutrient Analysis. Samples (0.4 g) were suspended in distilled water (1 mL) and nitric acid (8 mL; 65% (v/v)) and placed in specialized acid digest tubes for microwave-assisted digestion (MARS 6, CEM, Matthews, USA). Samples were heated in two temperature gradient: (1) from 20 °C to 150 °C over 15 min and (2) 150 °C ramp to 165 °C over 10 min and then held at this temperature for 20 min. The measured isotopes analyzed by ICP-MS were: ^{23}Na, ^{24}Mg, ^{31}P, ^{39}K, ^{44}Ca, ^{51}V, ^{52}Cr, ^{55}Mn, ^{56}Fe, ^{59}Co, ^{60}Ni, ^{63}Cu, ^{66}Zn, ^{78}Se , ^{95}Mo, ^{111}Cd, ^{202}Hg, ^{208}Pb. All the element standards were used in stock solutions of 1000 mg L^{-1}, which served for preparation of calibration solutions and internal standard solution. The decomposition matrix was nitric acid (2% (v/v)), hydrochloric acid (0.5% (v/v)) in distilled deionized water (Millipore, Bedford, MA) which was used for preparation of all the solutions. The ICP-MS measurements were done using Agilent 7700X spectrometer instrument (Agilent Technologies) equipped with a MicroMist nebulizer and nickel sampler and skimmer cones. The flow of standards and samples was joined together with a flow of Erbium internal standard solution (1 mg L^{-1}). The mixed flow (approximately 500 µL min^{-1}) was delivered by the peristaltic pump to the nebulizer of ICP-MS. Duration of ICP-MS analysis was 3.0 minutes. Data acquisition was one point, five replicates, 100 sweeps per replicate.

Phytochemical Analysis.

The phytochemicals were extracted by four methods specifically optimized for flavan-3-ols, anthocyanins, other flavonoids/isoflavanoids and phenolic acids/other phenolic compounds.

Flavanols. Samples (approx. 0.25 g dry weight) were suspended in a solvent extraction mixture (acetone/water/acetic acid, 70/28/2 (v/v/v); 5 mL), placed in an ultrasound bath for 10 min and the supernatant separated by centrifugation (5 min; 3220g; 4 ºC). The extraction was repeated twice, the supernatants combined and the pellet freeze-dried for alkaline hydrolysis. The solvent was removed from the supernatants by rotary evaporation at T ≤ 40 °C. The freeze-dried pellets were suspended in NaOH (3 mL; 1 mol dm^{-3}) and stirred at room temperature for four hours under nitrogen. The pH was reduced to pH 2 with HCl (10 mol dm^{-3}) and the samples extracted into EtOAc (5 mL) by shaking for five minutes. This procedure was repeated twice and the EtOAc extracts combined and the solvent removed by rotary evaporation at T ≤ 40 °C. All extracts were dissolved in methanol/water (50% (v/v); 2 mL) for Liquid Chromatography-Mass Spectrometry (LC-MS) analysis as detailed below.

Anthocyanins. Samples (0.1 g dry weight) were suspended in 1 mL of extraction mixture solvent (methanol/water/37%, HCl, 50/33/17 (v/v/v)) and placed in an ultrasound bath for 20 min. The supernatants were separated by centrifugation (5 min; 3220 x g; 4 ºC) and the remaining pellet freeze dried. The extraction was repeated twice and the supernatants combined and transferred to vials with a Teflon-lined screw cap. The vials were placed in a thermoblock and hydrolyzed at 100 °C for 60 min. Hydrolyzed samples were immediately cooled to room temperature, filtered on 0.2 µm filter and analyzed by High-Performance Liquid Chromatography (HPLC), as detailed below. The freeze-dried pellets were suspended in 3 mL of extraction mixture solvent (methanol/water/37% HCl, 50/33/17 (v/v/v)) and placed in an ultrasound bath for 20 min. The samples were transferred to vials with a Teflon-lined screw cap and hydrolyzed as described above.

Other flavonoids. Samples (1 g dry weight) were suspended in methanol/water (60/40 (v/v) containing 0.1% acetic acid; 8 mL) and placed on ultrasound bath for 60 min then the supernatant was separated by centrifugation (5 min; 3220g; 4 °C) and the pellet freeze dried.

The extraction was repeated twice and the supernatants combined. The solvent was removed under reduced pressure at a temperature not exceeding 40 °C. The dried extracts were suspended in HCl (4 mL; 1 mol dm^{-3}) and the samples incubated at 90 °C for one hour. After the acid hydrolysis the samples were extracted into EtOAc (10 mL) and the layers separated by centrifugation (5 mins; 3220g; 4 ºC). The extraction was repeated three times and the EtOAc extracts combined. The solvent was removed under reduced pressure at a temperature not exceeding 40 °C. The residue was dissolved in methanol (1 mL) and analyzed by LC-MS as detailed below. The freeze dried pellet was suspended in HCl (7 mL; 1 mol dm^{-3}), incubated at 90 °C for one hour and processed as described above.[28]

Phenolic acids and other phenols. Samples (approx. 0.1 g dry weight) were suspended in HCl (3 mL; 0.2 mol dm^{-3}) and extracted into EtOAc (5 mL) and the layers separated by centrifugation (5 min; 1800g; 4 ºC). The extraction was repeated twice and the EtOAc extracts combined. The organic layer was left to stand over sodium sulphate (anhydrous), filtered and the solvent removed under reduced pressure at a temperature not exceeding 40 °C. The remaining aqueous fraction, obtained after EtOAc extraction, was neutralized and freeze dried. The freeze-dried pellets were suspended in NaOH (3 mL; 1 mol dm^{-3}) and stirred at room temperature for four hours under nitrogen. The pH was reduced to pH 2 and samples extracted into EtOAc (5 mL). This was repeated twice and processed as described above. The pH of the aqueous fraction was then brought to pH 7 and the aqueous fraction was freeze dried. The freeze dried aqueous fractions were suspended in HCl (3 mL; 2 mol dm^{-3}) and incubated at 95 °C for 30 min with intermittent mixing. The samples were cooled and extracted with EtOAc (5 mL x 3) and processed as described above. All extracts were dissolved in methanol (1 mL) for LC-MS analysis as described below.[28]

Preparation of the extracts for LC-MS analysis. An aliquot (20 µL) of the each type of extract prepared above was transferred to an eppendorf. Internal standard 1 for negative mode mass spectrometry (IS1; ^{13}C benzoic acid; 2 ng μL^{-1} in 75% methanol containing 0.02% acetic acid; 20 µL), internal standard 2 for positive mode mass spectrometry (IS2; 2-amino-3,4,7,8-tetramethylimidazo[4,5-f] quinoxaline; 0.5 ng μL^{-1} in 75% methanol containing 0.02% acetic acid; 20 µL) and acidified (HCl; 0.4 mol dm^{-3}) methanol (40 µL) were added. The samples were mixed well, centrifuged (12,500g; 5 min; 4 ºC) and the supernatants analyzed by LC-MS as detailed below.

LC-MS Analysis. Liquid chromatography separation of the metabolites was performed on an Agilent 1100 LC-MS system from using a Zorbax Eclipse 5µm, 150 mm x 4mm column from Agilent Technologies (Wokingham, UK) as described elsewhere.[28,29] Three gradients were used to separate the different categories of metabolites and the mobile phase solvents in each case were water containing 0.1% acetic acid (A) and acetonitrile containing 0.1% acetic acid (B). Method 1: 40–90% B (13 min), 90% B (1 min), 90–40% B (1 min), 40% B (9 min); method 2: 10–55% B (45 min), 55–80% B (15 min), 80% B (3 min), 80–10% B (0.2 min), 10% B (4.8 min) and method 3: 50–80% B (10 min), 80% B (2 min), 80–50% B (1 min), 50% B (4 min). In all cases the flow rate was 300 µL min^{-1} with an injection volume of 5 µL. The LC eluent was directed into, without splitting, an ABI 3200 triple quadrupole mass spectrometer (Applied Biosystems, Warrington, UK) fitted with a Turbo Ion Spray™ (TIS) source. For LC methods 1 and 2, the mass spectrometer was run in negative ion mode with the following source settings: ion spray voltage -4500 V, source temperature 400 ºC, gases 1 and 2 set at 15 and 40 respectively and the curtain gas set to 10 (units). For LC method 3, the mass spectrometer was run in positive ion mode with the following source settings; ion spray voltage 5500 V, source temperature 400 °C, gases 1 and 2 set at 14 (units) and 40 (units),

respectively, and the curtain gas set at 10 (units). All the metabolites were quantified using multiple reaction monitoring (MRM). Standard solutions (10 ng μL^{-1}) for all analytes were prepared and pumped directly via a syringe pump. The ion transitions for each of the analytes were determined based upon their molecular ion and a strong fragment ion. For several categories of compounds, it was inevitable that their molecular ion and fragment ion would be the same, but this was overcome by their different elution times. Their voltage parameters; declustering potential, collision energy and cell entrance/exit potentials were optimized individually for each analyte.

HPLC Analysis. Liquid chromatography separation of the was performed on an Agilent 1200 HPLC system (Agilent Technologies, Wokingham, UK) equipped with binary pumps, thermostated autosampler, column oven and diode-array detector. The column used was a Synergi Polar-RP (250 x 4.6 mm; 4 µm i.d.; 80Å), the guard column (4 x 3 mm), both from Phenomenex (Macclesfield, UK). The mobile phase was 2.13% formic acid aqueous solution (A) and acetonitrile/methanol (B; 85/15 (v/v)) with an isocratic program of 45 min. Operating conditions were: column temperature (35 °C), injection volume (20 µL), flow rate (1 mL min^{-1}) with UV/VIS photo-diode array detection at 530 nm.

Statistical Analysis. All the analyses were performed in triplicate and are presented as the mean ± standard deviation. The statistical analysis on macro- and micro-nutrient contents was performed using SPSS 23.0 for Windows. The Shapiro–Wilk test was applied to verify the normal distribution of the variables. When the statistical distribution was not normal, a logarithmic transformation of the variables was performed. The Levene's test was applied to detect possible non-homogeneity of the variances. The data were analyzed using One-Way-

225 Analysis of Variance (ANOVA) to compare the groups and performed Tukey's test for
226 multiple comparisons. Data on phytochemicals were analyzed by principal component
227 analysis (PCA), unit variance (UV)-scaled using Sigma P^+ 12 (Umetrics, Cambridge).

RESULTS AND DISCUSSIONS

Macronutrient Composition. The macronutrient composition of the flours is shown in Table 1. The protein content varied, with the highest values observed for lupin (43.0 ± 0.17 % (w/w)) and hemp (38.55 ± 0.32 % (w/w)). Green pea and fava bean had similar protein contents (24.60 ± 0.09 and 22.12 ± 0.04 % (w/w) respectively), while buckwheat showed lower values (20.05 ± 0.05 % (w/w)). These observations, which are in line with previous reports,[30,31] were not unexpected, as the crops from the *Fabaceae* family such as lupin, green pea and fava bean, are known to be one of the best sources of plant protein. Conversely, buckwheat from the *Polygonaceae* family was expected to have lower protein content than legumes. Furthermore, the high protein content of hemp is well documented in the literate.[32] Also, previous research suggested that the crops had a good essential amino acids profile, even though some of them, mainly the legumes, may not provide the necessary amounts of sulphur-containing amino acids.[33-35] However, the amino acid deficiency, which is usually rare in Western diets, can be overcome by integrating legume-based dishes with cereal products, as the amino acid contents of the two kinds of crops are complementary with respect to human nutritional requirements.[36] Moreover, the protein content of the selected crops were all significantly higher ($p < 0.001$) that wheat and are well suited to supplement carbohydrate-based diets.[37] Thus, the selected crops may have potential to be applied as affordable local sources of dietary protein. All the selected crops had significantly higher ($p < 0.001$) dietary fibre content (Table 1) compared to wheat (0.55 ± 0.1 % (w/w)). In particular, lupin and hemp were shown to be the richest sources of insoluble fibre (> 23 % (w/w)), which due to its fermentative capacity can stimulate the growth of bifidobacteria, exerting prebiotic effects.[38] Moreover, since dietary fibre has been shown to be effective in lowering the blood cholesterol,[39] it is likely that the selected crops, particularly lupin and hemp, also in

253 the form of flour ingredients for bread, would merit consideration in the ongoing efforts to
254 design healthy foods, with potential to increase the excretion of fat and cholesterol, and
255 promote the production of short chain fatty acids with anti-inflammatory activities. The total
256 fat content was generally low (<4 % (w/w)) for the flours studied, apart from hemp and lupin
257 (12.46 ± 0.32 and 6.78 ± 0.34 % (w/w), respectively, which were significantly higher ($p <$
258 0.001). From the nutritional standpoint, when below 37%, fat quality is more important than
259 fat quantity, and it is acknowledged that lupin and hemp oils are characterized by a well-
260 balanced fatty acid composition with amounts of total unsaturated fatty acids much higher
261 than total saturated fatty acids.[40,41] Remarkably, lupin even though belonging to the *Fabaceae*
262 family like fava bean and green pea, showed levels of total fats notably higher than the other
263 crops. In this context, lupin and to some extent hemp could be potentially considered valuable
264 industrial crops, as partial replacement of meat with these plant products could result in a
265 reduction of less healthy saturated animal-derived fats. On the other hand, the higher level of
266 unsaturated fats, although beneficial, do come with inherent problems of rancidity
267 development. These values are in agreement with previous studies,[42] however, the
268 macronutrient composition of plant foods is likely to be strongly influenced by numerous
269 factors such as cultivar, environment and grade of processing.
270
271 **Micronutient Composition.** The results of the micronutrient mineral analysis of the flours
272 are presented in Table 2. All the flours generally contained a broad variety of minerals with
273 high levels of sodium, magnesium, phosphorous, potassium, calcium, manganese. Hemp
274 flour was significantly higher ($p < 0.001$) in sodium, magnesium, phosphorous, potassium
275 and calcium with values of 260.62 ± 21.00, 4340.91 ± 184.80, 9721.65 ± 413.39, 8572.49 ±
276 425.11, 1790.98 ± 76.36 mg kg^{-1}, respectively. Iron content ranged from 69.57 ± 7.43 mg kg^{-1}
277 in green pea to 11.62 ± 0.89 mg kg^{-1} in wheat. Fava bean and buckwheat resulted minor

sources of calcium (269.26 ± 7.57 and 267.77 ± 27.01mg kg^{-1}, respectively), as the RDI for calcium is 800 mg per day, while hemp and green pea resulted significant dietary sources of zinc (74.38 ± 3.30 and 64.51 ± 4.34 mg kg^{-1}, respectively), given its RDI of 15 mg per day. Manganese, and selenium were found in all the flours, even though at very low levels in some cases, and wheat had the lowest value for all the minerals except for calcium (1057.86 ± 20.53 mg kg^{-1}). Clearly there are variations in the mineral content detected compared with previous research.[43,44] Again, these variations are likely to be due to the use of different varieties, varying environmental conditions, such as soil characteristics and fertilizer applications, and to a lesser degree, method of detection. However, some general commonalities were observed, such as the concentrations of phosphorous in hemp and potassium in buckwheat. The dietary deficiencies of mineral elements can have detrimental effects on health, and it has been estimated that 3.7 billion people worldwide are ferrous deficient, even though other common mineral elements deficiencies are zinc, iodine, magnesium, calcium and selenium.[45] Unfortunately, common foods do not always have adequate mineral concentrations to meet the dietary requirements, hence, mineral fortifications are necessary. Therefore, the use of the selected flours to enhance the mineral contents of nutritionally poor food could be a cost effective approach to control some mineral deficiencies, e.g. the use of hemp flour to manage zinc deficiency. However, some antinutrients found in plants, such as phytic acid and tannins, must be inactivated or removed prior to mineral fortification.[44] The flour samples also showed exhibited traces of some heavy metals. The contamination may be due to certain anthropogenic activities, such as mining, urban development, the application of fertilizers and pesticides, but also to the milling process. However, their concentrations are all below the safe limits for adults.[46]

Phytochemical Composition. The principal component analysis (PCA) (Figure 1) regarding the phytochemical profiles of the selected crops (Tables 3-6), as measured by LC-MS, showed that green pea, fava bean, and wheat clustered, suggesting similar metabolite profiles. Comparing the individual metabolites, these crops had lower levels of benzoic acid derivatives (Table 6). Green pea and fava bean are both from the *Fabaceae* family, however, wheat (*Poaceae*) also shares the lower left-handed quadrant of the diagram and had the lowest content of benzoic acid and derivatives among the flours studied (19.95 ± 2.71 mg kg^{-1}). Even though lupin belongs to the *Fabaceae* family, unlike green pea and fava bean, it is not rich in benzoic acid and derivatives, and discriminates from the other two legume crops due to its distinctive flavonoid profile. It is the only crop to show sizeable amounts of genistein and tyrosol. The PCA plot also indicated that buckwheat and hemp discriminated from the other samples. Buckwheat presented a different metabolite profile due to the higher concentrations of flavonoids mainly quercetin (35.66 ± 2.22 mg kg^{-1}), whereas hemp had an overall higher content of phenolic acids (348.61 ± 20.28 mg kg^{-1}).

The cumulative sum of the phenolic compounds from the selected flours ranged between 0.1 and 1.3 g kg^{-1}, in the following order: buckwheat > fava bean > hemp > lupin > wheat > green pea. It should be noted that some metabolites such as phenylpyruvic acid could be derived from protein degradation,[47] and this may explain why levels are higher in lupin compared to the other crops analyzed, as it had the highest protein content (43.0 ±. 0.17 % (w/w)).

Table 3 shows the content of flavanols in the flours. Among the flours analyzed, the highest content was found in buckwheat 253.99 ± 35.41 mg kg^{-1} followed by fava bean 41.21 ± 1.74 mg kg^{-1}. The concentrations of catechin and epicatechin in both samples are higher in the free than bound form. Very low levels of catechin and epicatechin were found in hemp flour (1.66 ± 0.26 and 0.46 ± 0.14 mg kg^{-1} in the bound form, respectively). With regard to individual

compounds, epicatechin was the flavanol most abundant in the flours analyzed, gallocatechin, epigallocatechin, epigallocatechin gallate were not identified in any of the flours studied and catechin and epicatechin were not detected in lupin, green pea or wheat. The presence of low molecular weight flavanols in fava bean is confirmed in literature,[48] even though some variations are likely due to the impact of food processing on seeds (drying, milling, etc.) as a number of studies reported reduction in monomeric flavanols when exposed to high temperatures.[49,50] High levels of epicatechin were already identified in buckwheat extracts, but former studies focused their attention on the vegetative parts of the plant, such as leaves and flowers, which in some countries are consumed as a vegetable[51]. As human intervention studies clearly suggests that flavanols exert numerous beneficial effects, particularly on cardiovascular health,[52,53] the detection of high levels of flavanols in buckwheat after the milling process is noteworthy, as it enhances its applicability as functional ingredients in processed food products.

Table 3 shows the content of bound anthocyanins from the flours released after the acid hydrolysis. Pelargonidin was present in substantial amounts (748.17 ± 75.55 mg kg^{-1}) in buckwheat flour, even though cyanidin was the most common among the flours (29.72 ± 3.71 mg kg^{-1} in buckwheat, 23.15 ± 5.28 mg kg^{-1} in fava bean, 58.43 ± 21.01 mg kg^{-1} in hemp). Delphinidin was present at the lowest concentration amongst the anthocyanins detected (25.55 ± 1.27 mg kg^{-1} only in fava bean). As anthocyanins have shown to exert protective activities against cardiovascular disease and cancer when high concentrations were consumed,[54,55] there are still insufficient data to infer that the consumption of the selected crops could ameliorate some pathological conditions, while the role of anthocyanin rich fruits such as wild blackberries (anthocyanins content about 2800 mg kg^{-1})[56] against the deleterious effects of chronic diseases seems more convincing. There are only very few studies on the identification and quantification of anthocyanins in foods with no distinctive red, blue and

purple colours.[57,58] Therefore, the present work identifies novel anthocyanin rich crops with potential for industrial utilization.

The content of the other individual flavonoids in the grain flours is reported in Table 3. The bound flavonoids were released after acid hydrolysis. Buckwheat had the highest content of flavonoids (43.92 ± 2.24 mg kg^{-1}) followed by lupin (19.66 ± 0.39 mg kg^{-1}), fava bean (10.62 ± 0.52 mg kg^{-1}), green pea (7.05 ± 0.46 mg kg^{-1}) hemp (6.86 ± 1.17 mg kg^{-1}) and wheat (0.65 ± 0.06 mg kg^{-1}). The main flavonoids in flours were quercetin for buckwheat (35.66 ± 2.22 mg kg^{-1}), tyrosol for lupin and hemp (15.28 ± 0.33 and 3.80 ± 1.14 mg kg^{-1}, respectively), quercetin and kaempferol for fava bean 3.97 ± 0.16 and 3.49 ± 0.42 mg kg^{-1}, respectively), kampferol and tyrosol for green pea (2.74 ±0.24 and 2.11 ± 0.04 mg kg^{-1}, respectively). Quercetin was detected in all the samples, although only in buckwheat, and slightly in hemp, at relevant concentrations. Lupin was the only crop to show a significant content of tyrosol. Over the last decades, flavonoids have earned great scientific attention due to their beneficial effects and their ubiquity in plant foods. Therefore, a wealth of research has been carried out on identification of dietary sources of flavonoids, and some products such as spinach, cauliflower and onions resulted particularly abundant in flavonoids.[59] However, up to date most of the studies have based their investigations on raw food, even though the human diet includes plenty of cooked and processed products. Contrary, the selected crops stand out as flavonoid rich new ingredients, as being in the form of flours, they are already available to be included in formulated products, and offer the opportunity to improve the nutritional characteristics of processed food delivering flavonoid compounds, mainly quercetin, kampferol and tyrosol.

The content of phenolic acids and derivatives from the selected flours are shown in Table 4, and ranged from 50 to 350 mg kg^{-1} in the following order: hemp > fava bean > buckwheat > wheat > green pea > lupin. The 3-hydroxyphenylacetic acid in bound form was the main

phenolic acid found in buckwheat (63.64 ± 36.16 mg kg^{-1}), bound ferulic acid was the main phenolic acid in green pea, fava bean and wheat flours (12.11 ± 0.58, 229.51 ± 36.58, 60.21 ± 3.64 mg kg^{-1}, respectively), bound *p*-coumaric acid was the main phenolic acid in hemp flour (82.78 ± 8.09 mg kg^{-1}), while bound 4-hydroxyphenylpyruvic acid was the main phenolic acid in lupin flour (25.15 ± 4.16 mg kg^{-1}). Benzoic, salicylic, protocatechuic, vanillic and ferulic acids were reported in both free and bound forms in all the flours, whereas gallic acid was not detected in lupin and wheat and syringic and caffeic acids were not detected in lupin. As ferulic dimers are widely acknowledged as compounds with strong antioxidant activity,[60] noteworthy were the amounts of bound 5-5´ and 8-5 linked ferulic acid in fava bean flour. Human intervention studies showed that high plasmatic concentration of lignans were associated with lower risk of colon cancer.[61] Therefore, noteworthy are the amounts of bound secoisolariciresinol and syringaresinol in hemp flour, the use of which might be efficacious to increase the intake of dietary lignans, which ranges between 0.15 and 1.1 mg per day in Western diets.[61] As the content and composition of phenolic acids is highly dependent on variables such as species and variety, growth conditions, extend of development, harvest time, type of soil and method of detection, it was not always possible to match the values found in the previous literature, however, some trends have been identified, such as high levels of p-coumaric and ferulic acids in fava bean,[62] and the significant concentrations of protocatechuic acid in buckwheat.[63]

The detailed analysis on the phenolic composition of selected crops in this study suggests that phenolic acids are mainly found attached to other plant components, most likely polymers of the cell wall and this is in agreement with previous studies.[29,64] It is very likely that the bound phenolic compounds, which constitute the major phenolic fraction in legumes and cereals,[65] are delivered to the colon, where they are available for metabolism by the gut microbiota.

However, this may not be the case of other phenolic compounds such as the flavanols, which were detected mainly in the free form.

Buckwheat, green pea, fava bean, hemp and lupin have a valuable macronutrient, micronutrient and non-nutrient (phytochemical) profile with significant potential to benefit human health. The favourable macro- and micronutrient profile of the flours suggests an important role in human nutrition as their use could range from being staples in the diet to new low-cost ingredients for healthier reformulations of processed foods. Indeed the shift towards a better diet by improving the basal raw materials in food and reformulation is a common message at global, national, and devolved government and industry levels.[66] The selected crops, being rich in protein, could also be considered alternative choices to soybean and meat, the production of which is responsible for a significant level of agri-food-related environmental pressure (e.g. GHG emissions). Their implementation or supplementation into the human diet is a possible approach to satisfy the global demand for protein in a sustainable manner. From an industrial standpoint, when used as food ingredients, they provide opportunities to enrich food considered nutritionally poor, through the development of functional and healthier products, the market share of which is quickly growing, as in Western Europe more than 50% of consumers embraces the fortification of food products with functional ingredients.[67] The health benefits, beyond the nutritional enhancement, are likely to be due to their favourable phytochemical profile, as a wide range of phenolic compounds was detected. Some of the phenolic compounds were found at significant concentrations, among them: p-hydroxybenzoic acid, protocatechuic acid, gallic acid, p-coumaric acid, catechin and epicatechin, which are known for their anti-inflammatory activities.[68] Also, the majority of the phytochemicals were detected in the bound form, particularly the phenolic acids. Their association to dietary fibre brings further beneficial opportunities, due to their beneficial impact on colonic bacteria. Furthermore, from a purely

industry processing perspective the recognized antioxidant activity of the phenolic compounds identifies the selected crops (and their associated products such as flour, etc) as being imbued with inherent natural food preservative ability, potentially allowing for a reduction in artificial antioxidant additives. However, their incorporation into foods will undoubtedly impact on sensorial features of the final products and further studies on their uses as food ingredient are required. Also, the prospect of sourcing plant protein from species (and varieties) adapted to the local climate, offers new opportunities for growers. In fact the diversification of the range of crops available to growers would also deliver into the "greening" requirements of the EU Common Agricultural Policy by crop diversification.[69] Therefore, the numerous health, environmental, and economic benefits, derived by their use, especially in high latitude countries where they offer resilience to the harder growing conditions, warrants further research initiatives.

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Notes

Authors declare no conflict of interests.

ABBREVIATIONS USED

CVD, cardiovascular disease

DAD, diode array detector

ESADI, estimated safe and adequate intake

EtOAc, ethyl acetate

GHGEs, greenhouse gas emissions

HPLC, high pressure liquid chromatography

ICP-MS, inductively coupled plasma-mass spectrometry

LC-MS, liquid chromatography-mass spectrometry

LDL, low-density lipoproteins

MRM, multiple reaction monitoring

NSP, non-starch polysaccharides

PCA, principal component analysis

RDI, recommended daily intake

UV, unit variance

UV/VIS, Ultraviolet–visible

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FIGURE CAPTIONS

Figure 1. Principal component analysis (unit variance-scaled) plot of the t(1) and t(2) axis showing discrimination of the crops based on all the phytochemicals measured.

659 **TABLES**

660 **Table 1. Macronutrient composition of the flours**

661

Sample	Dry Matter % (w/w)	Ash % (w/w)	Protein % (w/w)	Fat % (w/w)	Resistant Starch % (w/w)	Fibre % (w/w)	
						soluble	insoluble
green pea	89.30±0.09[a]	2.79±0.13[a]	24.60±0.09[a]	2.11±0.07[a]	0.59±0.01[a]	0.08±0.03[a]	8.69±0.07[a]
buckwheat	89.89±0.11[b]	3.42±0.10[a,c]	20.05±0.05[b]	1.58±0.25[a]	0.33±0.01[b,d]	0.82±0.16[a,d]	6.98±0.01[a]
hemp	91.95±0.16[c]	10.59±0.55[b]	38.55±0.32[c]	6.78±0.34[b]	n/d	0.16±0.00[b,e]	25.49±1.45[b]
lupin	92.86±0.08[d]	3.65±0.05[c,d]	43.00±0.17[d]	12.46±0.32[c]	0.03±0.01[c]	1.61±0.08[c]	23.55±1.10[c]
fava bean	91.19±0.07[e]	2.76±0.00[a]	22.12±0.04[e]	3.98±0.32[d]	0.34±0.05[d]	0.56±0.10[d]	9.39±0.30[a]
wheat	88.02±0.09[f]	4.84±1.19[d]	13.57±0.09[f]	1.59±0.25[a]	0.25±0.00[e]	0.25±0.03[e]	0.30±0.07[d]

662 Data are means of three replicates with standard deviations. n/d = not detected (i.e. below the detection level). [a-f]Data within the same column
663 with different superscripts are significantly different ($p < 0.05$).

664 **Table 2. Micronutrient composition of the flours**

Sample	buckwheat	green pea	fava bean	hemp	lupin	wheat	RDI, ESADI & PTI
Na	138.72± 11.73 [a]	181.31± 8.32 [a]	224.29± 11.08 [b]	260.62± 21.00[c]	142.38± 14.22[a]	73.35d± 21.76[d]	1100-3300[1]
Mg	1608.41± 96.66[a]	3813.58± 206.01[b]	545.86± 13.25[c]	4340.91± 184.80[d]	947.82± 80.75[e]	173.76± 6.68[f]	300-350[2]
P	2770.98± 171.18[a]	8617.55± 545.99[b]	2219.86± 48.62[a]	9721.65± 413.39[c]	2667.65± 300.89[a]	721.62± 23.00[d]	800[2]
K	3384.02± 208.93a	7450.66± 443.42[b]	5496.49± 141.26[c]	8572.49± 425.11[d]	7008.94± 621.54[b]	1045.23± 30.42[e]	1875-5625[1]
Ca	267.27± 27.01[a]	1540.40± 83.87[b,c]	269.26± 7.57[a]	1790.98± 76.36[c]	1062.74± 109.14[d,e]	1057.86± 20.53e	800[2]
Mn	13.08± 0.73[a]	108.30± 8.12[b,c]	5.28± 0.12[a]	128.00± 7.72[c]	627.11± 50.17[d]	5.44± 0.19[a]	2.5-5.0[1]
Fe	20.23± 0.86[a]	69.57± 7.43[b,c]	16.36± 0.47[a]	51.91± 10.22[c]	32.15± 22.48[a,c]	11.62± 0.89[a]	10-18[2]
Co	n/d	0.08± 0.04	0.05± 0.01	0.16± 0.09	0.20± 0.18	n/d	0.003[3]
Cu	3.81± 0.21[a]	13.61± 0.91[b]	3.97± 0.18[a]	15.58± 1.36[c]	5.99± 0.08[d]	1.11± 0.12[e]	2-3[1]
Zn	18.50± 0.43[a]	64.51± 4.34[b]	20.49± 0.99[a]	74.38± 3.30[c]	36.54± 1.00[d]	5.76± 0.16[e]	15[2]
Se	0.09± 0.03[a]	0.10± 0.00[a]	0.07± 0.00[a]	0.11± 0.02[a]	0.60± 0.12[b]	0.04± 0.02[a]	0.05-0.2[1]
Mo	0.25± 0.01[a]	0.44± 0.04[b,d]	1.45± 0.04[c]	0.47± 0.00[d,e]	0.61± 0.14[e]	0.08± 0.00[f]	0.15-0.5[1]
Cd	0.03± 0.00[a]	0.03± 0.00[a,c]	0.01± 0.00[b,d]	0.04± 0.00[c]	0.01± 0.01[d,e]	0.01± 0.00[e]	0.001[4]
Hg	n/d	n/d	n/d	n/d	0.02± 0.00	0.01± 0.00	0.0007[4]
Pb	0.05± 0.02	0.06± 0.04	0.04± 0.00	0.09± 0.07	0.12± 0.09	0.03± 0.01	0.007[4]

665 Data are means of three replicates with standard deviations and is expressed as mg kg^{-1} dry weight. n/d = not detected (i.e. below the detection
666 level). [a-f]Data within the same row with different superscripts are significantly different ($p < 0.05$). [1]Estimated Safe and Adequate Dietary Intake
667 (ESADI); [2]reference daily Intake (RDI); [3]expressed as weight of vitamin B_{12} and [4]provisional tolerable intakes (PTI), expressed as mg kg^{-1}
668 body weight.

669 **Table 3. Flavonoids (A), coumarins (B) and isoflavonoid (C) flavanol (D) and anthocyanin (E) content of the flours**

	buckwheat	green pea	fava bean	hemp	lupin	wheat
A						
bergapten	0.03± 0.00	0.03± 0.00	0.03± 0.00	0.01± 0.00	0.02± 0.01	0.05± 0.00
coumestrol	n/d	0.02± 0.00	n/d	n/d	n/d	n/d
isoliquiritigenin	0.01± 0.00	0.01± 0.00	0.01± 0.00	n/d	n/d	n/d
phloretin	0.07± 0.02	n/d	n/d	n/d	n/d	n/d
naringenin	0.49± 0.06	0.09± 0.05	0.11± 0.01	0.01± 0.00	0.16± 0.02	n/d
hesperitin	0.04± 0.01	n/d	0.05± 0.00	0.01± 0.01	0.06± 0.05	0.03± 0.01
kaempferol	0.65± 0.11	2.74± 0.24	3.49± 0.42	0.04± 0.01	0.09± 0.02	0.07± 0.01
morin	n/d	n/d	n/d	0.08± 0.01	0.03± 0.05	n/d
quercetin	35.66± 2.22	1.08± 0.36	3.97± 0.16	0.73± 0.14	0.02± 0.01	0.11± 0.02
myricetin	0.05± 0.00	0.04± 0.04	1.31± 0.24	n/d	n/d	0.01± 0.00
quercetin-3-glucoside	0.66± 0.04	0.03± 0.00	0.17± 0.02	n/d	n/d	0.01± 0.00
taxifolin	0.28± 0.02	0.48± 0.11	0.18± 0.03	0.08± 0.03	n/d	n/d
scopoletin	0.06± 0.00	0.04± 0.01	0.01± 0.00	0.26± 0.06	n/d	n/d
hesperidin	0.02± 0.00	0.02± 0.00	0.01± 0.00	0.02± 0.00	0.01± 0.00	0.02± 0.01

quercitrin	n/d	0.04± 0.06	n/d	n/d	n/d	n/d
poncirin	n/d	0.01± 0.00	0.02± 0.00	n/d	n/d	n/d
didymin	0.01± 0.00	n/d	n/d	n/d	n/d	n/d
phloridzin	n/d	n/d	n/d	n/d	0.01± 0.01	n/d
galangin	n/d	n/d	0.01± 0.00	n/d	0.05± 0.01	n/d
luteolin	1.10± 0.03	n/d	0.10± 0.01	0.63± 0.16	0.44± 0.06	0.03± 0.00
fisetin	n/d	0.01± 0.01	0.01± 0.00	n/d	n/d	n/d
luteolinidin	0.21± 0.01	n/d	n/d	n/d	n/d	n/d
isorhamnetin	0.57± 0.05	0.09± 0.01	0.43± 0.03	0.19± 0.04	0.02± 0.00	0.24± 0.05
apigenin	0.08± 0.01	0.01± 0.00	0.06± 0.01	0.17± 0.03	0.69± 0.03	0.02± 0.00
tyrosol	1.73± 0.25	2.11± 0.04	0.06± 0.01	3.80± 1.14	15.28± 0.33	n/d
hydroxityrosol	0.02± 0.00	n/d	0.15± 0.02	0.08± 0.05	0.01± 0.01	n/d
B						
coumarin	n/d	0.03± 0.03	0.01± 0.01	0.39± 0.03	0.02± 0.00	n/d
7,8-dihydroxy-6-methylcoumarin	n/d	n/d	0.02± 0.02	n/d	n/d	n/d
umbelliferone	0.24± 0.01	n/d	0.01± 0.00	0.05± 0.01	n/d	n/d

psoralen	0.04± 0.00	n/d	n/d	n/d	n/d	n/d
C						
biochanin A	n/d	n/d	0.15± 0.01	0.12± 0.03	0.02± 0.02	0.01± 0.00
daidzein	n/d	n/d	n/d	n/d	0.04± 0.02	n/d
genistein	0.01 0.00	n/d	n/d	n/d	2.37± 0.16	0.01± 0.01
formononetin	0.03± 0.01	0.05± 0.01	0.05± 0.02	0.03± 0.00	0.05± 0.05	0.02± 0.00
D						
catechin	68.93± 13.02	n/d	13.18± 0.45	1.66± 0.26	n/d	n/d
epicatechin	185.06± 33.42	n/d	28.03 ± 1.71	0.46± 0.14	n/d	n/d
E						
delphinidin	n/d	n/d	25.55± 1.27	n/d	n/d	n/d
cyanidin	29.72± 3.71	n/d	23.15± 5.28	58.43± 21.01	n/d	n/d
pelargonidin	748.17± 75.55	n/d	n/d	n/d	n/d	n/d

Data are means of three replicates with standard deviations and is expressed mg kg^{-1} dry weight. n/d = not detected (i.e. below the detection level). 8-methylpsoralen, tangeretin, eriocitrin, naringin, neoeriocitrin, neohesperidin, gossypin, gallocatechin, epigallocatechin, epigallocatechin gallate, peonidin, malvinidin, and petunidin were not detected in any of the samples.

673 **Table 4. Phenolic acids and other related compounds:** Benzoic acids, aldehydes and acetophenones (A), cinnamic acids and alcohols (B),
674 phenylacetic, phenylpyruvic and phenyllactic and phenylpropionic acid (C) simple phenols and nitrogen containing compounds (D) and phenolic
675 dimers and lignans (E)

	buckwheat		green pea		fava bean		hemp		lupin		wheat	
	free	**bound**	**free**	**bound**	**free**	**bound**	**free**	**bound**	**free**	**bound**	**free**	**bound**
A												
benzoic acid	1.41± 0.12	10.78± 1.44	1.27± 0.13	2.48± 0.13	0.82± 0.19	2.94± 0.67	2.79± 0.16	3.57± 0.22	1.39± 0.15	6.53± 0.70	1.41± 0.28	4.37± 0.61
salicylic acid	0.88± 0.11	4.02± 1.95	0.13± 0.02	0.27± 0.03	0.63± 0.06	0.44± 0.05	13.56± 0.26	4.33± 0.27	0.20± 0.01	2.22± 0.66	0.12± 0.01	0.61± 0.14
m-hydroxybenzoic acid	5.75± 0.16	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d
p-hydroxybenzoic acid	1.55± 0.12	21.41± 7.17	1.03± 0.07	8.25± 0.65	0.72± 0.06	7.54± 0.67	5.20± 0.13	13.31± 0.76	0.89± 0.28	10.18± 1.92	0.14± 0.01	0.99± 0.28
2,3-dihydroxybenzoic acid	0.15± 0.01	6.11± 3.55	0.01± 0.02	0.05± 0.06	0.08± 0.02	0.15± 0.03	0.12± 0.01	0.22± 0.05	0.04± 0.01	n/d	n/d	0.04± 0.01
2,4-dihydroxybenzoic acid	n/d	0.18± 0.04	n/d	n/d	n/d	n/d	n/d	0.10± 0.09	n/d	n/d	0.19± 0.01	n/d
gentisic acid	0.25± 0.05	9.98± 5.91	0.10± 0.10	0.82± 0.17	0.19± 0.01	2.18± 0.28	0.48± 0.08	31.20± 1.67	n/d	0.54± 0.11	n/d	0.17± 0.03
2,6-dihydroxybenzoic acid	n/d	0.14± 0.04	n/d	n/d	0.30± 0.02	0.04± 0.01	0.26± 0.01	0.03± 0.00	n/d	n/d	n/d	n/d
protocatechuic acid	6.61± 0.75	24.56± 7.80	1.26± 0.02	11.38± 1.18	0.61± 0.12	2.42± 0.26	5.63± 0.10	22.06± 2.13	0.15± 0.02	n/d	0.07± 0.01	0.11± 0.04
3,5-dihydroxybenzoic acid	n/d	n/d	n/d	n/d	n/d	0.79± 0.36	n/d	n/d	n/d	n/d	n/d	n/d
o-anisic acid	6.00± 0.10	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d
m-anisic acid	n/d	0.18± 0.04	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d

	buckwheat		green pea		fava bean		hemp		lupin		wheat	
	free	**bound**	**free**	**bound**	**free**	**bound**	**free**	**bound**	**free**	**bound**	**free**	**bound**
p-anisic acid	0.14± 0.05	0.76± 0.23	n/d	0.10± 0.04	0.16± 0.02	0.16± 0.07	0.04± 0.07	0.37± 0.02	n/d	0.28± 0.04	0.08± 0.07	0.46± 0.03
gallic acid	3.87± 0.43	30.88± 9.27	n/d	0.17± 0.02	0.44± 0.13	0.85± 0.19	0.14± 0.03	0.30± 0.16	n/d	n/d	n/d	n/d
vanillic acid	0.38± 0.02	5.61± 2.54	0.62± 0.03	4.14± 0.51	0.55± 0.07	4.72± 0.38	2.33± 0.35	13.74± 1.31	0.55± 0.17	9.09± 1.93	0.34± 0.05	2.64± 0.19
syringic acid	0.12± 0.03	1.40± 0.56	0.13± 0.01	0.45± 0.01	0.35± 0.01	3.06± 0.47	0.62± 0.03	6.41± 0.70	n/d	n/d	0.06± 0.02	1.68± 0.24
3,4-dimethoxybenzoic acid	n/d	0.42± 0.11	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d
p-hydroxybenzaldehyde	0.59± 0.08	8.14± 3.60	0.10± 0.03	0.40± 0.06	0.45± 0.04	1.11± 0.24	7.16± 0.35	12.10± 0.96	0.42± 0.17	4.43± 0.43	0.12± 0.02	1.02± 0.12
protocatechualdehyde	3.65± 0.36	19.74± 6.05	0.12± 0.12	0.07± 0.03	0.68± 0.09	5.63± 0.47	6.41± 0.24	34.77± 5.15	n/d	n/d	n/d	0.19± 0.04
3,4,5-trihydroxybenzaldehyde	n/d	0.09± 0.08	n/d	n/d	1.09± 0.29	5.97± 1.64	0.05± 0.04	0.40± 0.24	n/d	n/d	n/d	n/d
vanillin	0.32± 0.05	2.73± 1.30	0.14± 0.03	0.47± 0.11	1.41± 0.16	2.05± 0.33	3.07± 0.16	24.56± 1.41	0.29± 0.06	5.28± 0.58	0.86± 0.09	2.66± 0.34
syringin	0.07± 0.01	0.80± 0.36	n/d	0.46± 0.06	0.16± 0.02	0.40± 0.07	1.44± 0.10	13.17± 0.78	n/d	n/d	0.05± 0.00	0.57± 0.07
4-hydroxyacetophenone	n/d	0.18± 0.07	n/d	0.04± 0.00	0.03± 0.01	0.09± 0.02	0.04± 0.00	0.40± 0.02	0.10± 0.02	0.73± 0.14	n/d	0.19± 0.03
4-hydroxy-3-methoxyacetophenone	n/d	0.17± 0.08	n/d	0.09± 0.02	0.08± 0.02	0.28± 0.07	0.06± 0.02	0.67± 0.08	0.03± 0.01	0.34± 0.02	0.03± 0.00	0.38± 0.03
4-hydroxy-3,5-dimethoxyacetophenone	n/d	n/d	n/d	0.10± 0.01	n/d	0.20± 0.06	n/d	0.43± 0.04	n/d	n/d	n/d	1.02± 0.13
3,4,5-trimethoxyacetophenone	n/d	0.01± 0.00	n/d	n/d	n/d	n/d	n/d	n/d	n/d	0.01± 0.00	n/d	0.01± 0.00
B												

	buckwheat		green pea		fava bean		hemp		lupin		wheat	
	free	bound	free	bound	free	bound	free	bound	free	bound	free	bound
cinnamic acid	0.69± 0.07	1.69± 0.07	0.50± 0.03	1.02± 0.07	0.60± 0.02	1.12± 0.07	0.78± 0.05	1.04± 0.07	0.93± 0.05	2.14± 0.40	0.09± 0.00	0.20± 0.02
o-coumaric acid	n/d	n/d	n/d	n/d	n/d	0.61± 0.53	n/d	n/d	n/d	2.86± 0.65	n/d	2.23± 0.27
m-coumaric acid	1.26± 0.06	4.36± 3.22	n/d	n/d	n/d	n/d	n/d	5.06± 1.00	n/d		n/d	n/d
p-coumaric acid	n/d	8.85± 1.51	1.10± 0.03	n/d	1.84± 0.35	20.32± 3.31	1.24± 0.32	82.78± 8.09	n/d	n/d	n/d	1.44± 0.11
caffeic acid	0.31± 0.02	41.74± 22.54	n/d	0.22± 0.01	0.14± 0.01	2.24± 0.34	0.15± 0.02	2.39± 0.81	n/d	n/d	n/d	0.42± 0.06
chlorogenic acid	1.19± 0.11	n/d	n/d	n/d	0.05± 0.01	n/d	1.70± 0.02	n/d	0.03± 0.06	n/d	n/d	n/d
ferulic acid	0.16± 0.00	5.38± 0.58	1.07± 0.09	12.11± 0.58	1.74± 0.31	229.51± 36.58	1.24± 0.06	20.65± 1.23	0.35± 0.11	3.34± 1.20	0.63± 0.34	60.21± 3.64
sinapic acid	0.26± 0.03	8.93± 1.08	0.26± 0.05	11.02± 0.82	0.25± 0.06	16.41± 2.83	0.08± 0.08	3.27± 0.40	n/d	n/d	n/d	14.70± 1.68
3,4-dimethoxycinnamic acid	n/d	0.17± 0.03	n/d	n/d	n/d	0.45± 0.11	n/d	n/d	n/d	n/d	n/d	n/d
3,4,5-trimethoxycinnamic acid	0.09± 0.01	1.15± 0.13	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	0.01± 0.00
coniferyl alcohol	n/d	n/d	n/d	0.03± 0.01	n/d	0.03± 0.01	n/d	n/d	n/d	n/d	n/d	n/d
C												
phenylacetic acid	0.49± 0.01	2.25± 0.42	0.47± 0.02	0.25± 0.02	0.40± 0.03	0.76± 0.10	0.39± 0.05	0.42± 0.05	0.55± 0.04	1.42± 0.27	0.30± 0.05	0.48± 0.13
3-hydroxyphenylacetic acid	0.86± 0.13	63.64± 36.16	n/d	0.47± 0.03	0.36± 0.05	4.29± 0.49	0.47± 0.07	3.44± 0.32	n/d	1.41± 0.21	n/d	0.30± 0.26
3,4-dihydroxyphenylacetic acid	n/d	0.71± 0.26	n/d	n/d	n/d	0.52± 0.05	n/d	n/d	n/d	n/d	n/d	n/d

	buckwheat		green pea		fava bean		hemp		lupin		wheat	
	free	bound	free	bound	free	bound	free	bound	free	bound	free	bound
4-hydroxy-3-methoxyphenylacetic acid	n/d	9.63± 5.84	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d
4-methoxyphenylacetic acid	n/d	0.05± 0.00	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d
3,4-dihydroxymandelic acid	0.19± 0.02	0.73± 0.17	n/d	n/d	0.15± 0.14	0.13± 0.12	0.62± 0.09	0.79± 0.22	0.04± 0.08	n/d	0.06± 0.00	0.04± 0.01
4-hydroxy-3-methoxymandelic acid	n/d	n/d	n/d	n/d	n/d	n/d	n/d	1.51± 0.08	n/d	n/d	n/d	n/d
phenylpyruvic acid	0.14± 0.02	0.11± 0.03	0.06± 0.01	0.12± 0.06	0.20± 0.02	0.23± 0.05	0.22± 0.02	0.22± 0.03	0.09± 0.01	0.43± 0.05	0.37± 0.05	0.86± 0.07
4-hydroxyphenylpyruvic acid	10.30± 1.50	10.37± 5.37	11.59± 3.42	11.83± 2.76	12.22± 1.49	11.45± 3.46	8.61± 0.72	11.87± 6.54	7.85± 1.40	25.15± 4.16	1.23± 0.24	5.96± 2.55
phenyllactic acid	0.18± 0.02	3.38± 1.55	0.74± 0.04	0.24± 0.03	0.67± 0.05	0.30± 0.03	0.23± 0.02	0.17± 0.08	0.19± 0.03	0.08± 0.01	0.05± 0.01	0.16± 0.03
4-hydroxyphenyllactic acid	n/d	6.65± 1.24	0.51± 0.18	n/d	0.80± 0.04	1.06± 0.44	0.26± 0.23	1.81± 0.21	n/d	n/d	n/d	0.30± 0.11
3-hydroxyphenylpropionic acid	n/d	n/d	n/d	n/d	n/d	n/d	n/d	0.24± 0.05	n/d	n/d	n/d	n/d
4-hydroxyphenylpropionic acid	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d
3,4-dihydroxyphenyl propionic acid	n/d	n/d	n/d	n/d	n/d	0.11± 0.09	n/d	0.46± 0.07	n/d	n/d	n/d	n/d
4-hydroxy-3-methoxy phenylpropionic acid	n/d	0.17± 0.11	n/d	0.19± 0.02	n/d	0.64± 0.05	0.14± 0.03	1.02± 0.10	n/d	1.63± 0.37	n/d	0.27± 0.04
3-methoxyphenylpropionic acid	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d
D												
phenol	4.14± 1.47	24.89± 13.13	1.69± 0.04	2.93± 0.54	4.03± 0.61	3.12± 0.54	56.42± 6.88	23.95± 2.06	n/d	n/d	n/d	1.29± 1.12

	buckwheat		green pea		fava bean		hemp		lupin		wheat	
	free	bound	free	bound	free	bound	free	bound	free	bound	free	bound
1,2-hydroxybenzene	n/d	4.16± 2.46	n/d	n/d	0.05± 0.09	n/d	n/d	n/d	n/d	n/d	n/d	n/d
1,3-hydroxybenzene	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d
1,2,3-trihydroxybenzene	n/d	n/d	n/d	n/d	0.28± 0.09	n/d	n/d	n/d	n/d	n/d	n/d	n/d
4-ethylphenol	n/d	n/d	n/d	n/d	n/d	0.01± 0.00	n/d	n/d	n/d	n/d	n/d	n/d
4-methylcatechol	0.01± 0.00	0.08± 0.03	n/d	0.11± 0.01	0.01± 0.00	0.09± 0.02	n/d	0.50± 0.08	n/d	0.37± 0.07	n/d	0.88± 0.05
anthranilic acid	0.05± 0.01	0.68± 0.19	0.03± 0.01	0.17± 0.05	0.04± 0.01	0.14± 0.01	0.16± 0.01	0.88± 0.27	n/d	0.51± 0.10	n/d	0.10± 0.02
quinaldic acid	0.14± 0.01	0.17± 0.06	n/d	0.02± 0.00	0.03± 0.00	0.17± 0.04	0.01± 0.00	0.01± 0.00	0.03± 0.00	0.03± 0.01	n/d	0.02± 0.00
E												
ferulic dimer (5-5 linked)	n/d	0.09± 0.02	n/d	0.07± 0.02	n/d	39.99± 1.10	n/d	0.02± 0.02	n/d	n/d	n/d	3.32± 0.46
ferulic dimer (8-8 linked)	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d
ferulic dimer (8-5 linked)	n/d	0.39± 0.11	n/d	0.19± 0.08	0.04± 0.03	58.17± 6.68	n/d	1.20± 0.22	n/d	n/d	n/d	n/d
secoisolariciresinol	0.11± 0.01	0.14± 0.08	n/d	n/d	0.06± 0.00	0.02± 0.00	0.04± 0.01	3.71± 0.18	n/d	n/d	n/d	0.22± 0.01
matairesinol	n/d	n/d	n/d	n/d	n/d	n/d	n/d	0.03± 0.01	n/d	n/d	n/d	n/d
enterodiol	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	0.04± 0.01	0.01± 0.00	n/d	n/d
enterolactone	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	0.16± 0.01	0.09± 0.01	n/d	n/d
syringaresinol	1.40± 0.11	2.12± 0.70	n/d	n/d	0.86± 0.05	0.52± 0.07	0.50 ± 0.44	24.86± 2.16	n/d	n/d	n/d	n/d

	buckwheat		green pea		fava bean		hemp		lupin		wheat	
	free	**bound**	**free**	**bound**	**free**	**bound**	**free**	**bound**	**free**	**bound**	**free**	**bound**
pinoresinol	0.26± 0.03	0.07± 0.06	n/d	n/d	0.14± 0.01	n/d	0.35± 0.03	1.64± 0.11	n/d	n/d	n/d	n/d
lariciresinol	0.29± 0.04	n/d	n/d	n/d	n/d	n/d	n/d	n/d	0.30± 0.07	n/d	n/d	n/d

Data are means of three replicates with standard deviations and is expressed mg kg^{-1} dry weight. n/d = not detected (i.e. below the detection level). The following compounds were not detected in any of the samples: 3-methoxybenzaldehyde, 3,4-dimethoxybenzaldehyde, 3,4,5-trimethoxybenzaldehyde, 2-hydroxycinnamyl alcohol, isovanillin, ellagic acid, 3-methoxycinnamic acid, 4-methoxycinnamic acid, 4-hydroxy-3-methoxycinnamyl alcohol, phenylpropionic acid, 2-hydroxyphenylpropionic acid, 3,4-dimethoxyacetophenone, 4-hydroxyphenylacetic acid, mandelic acid, 3-hydroxymandelic acid, 4-hydroxymandelic acid, 4-hydroxyphenylpropionic acid, ethylferulate, 5-hydroxymatairesinol.

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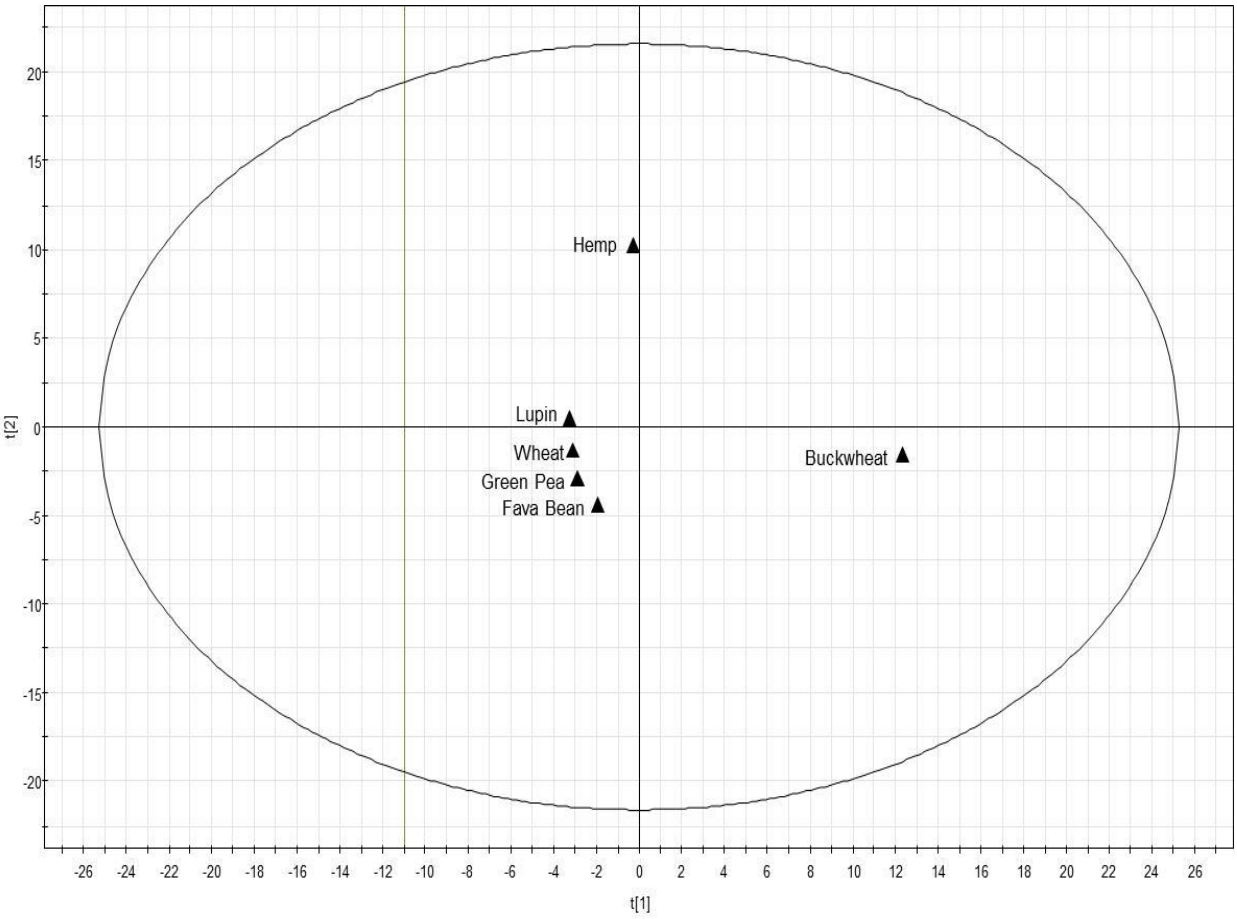

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